Supporting Information

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SI Text

Determination of Transgene Expression Levels. For each transgenic construct, multiple transgenic lines were established and expression levels were determined by Western blot analysis on protein extracts from heads of elav-GAL4 > *UAS-YARS* flies or by quantitative RT-PCR on RNA extracts from actin5C-GAL4^{weak} > *UAS-dYARS* flies. For Western blot analysis, a monoclonal anti-TyrRS antibody (Abnova) was used for TyrRS detection and monoclonal anti-α-tubulin antibody 12G10 (DSHB) was used as loading control. Band intensities were quantified using ImageJ software and transgenic lines with comparable expression levels were selected for further experiments (Fig. S2). For quantitative real-time PCR analysis, RNA was extracted from 2 experimental (actin5C-GAL4^{weak} > *UAS-dYARS*) and 2 control (TM6B > *UAS-dYARS*) groups for each transgenic line. cDNA was synthesized using the Transcriptor first strand cDNA synthesis kit (Roche) and qPCR was performed on an ABI PRISM 7000 cycler using *dYARS* primers (forward: 5′-GAGAAGTACATCAACCGACTGCTAGA-3′; reverse: 5′-GTTTTTGCAGTTCTGGGTTTTCA-3′) and RPII140 primers as an internal reference (forward: 5′-TTCCCCGATCACAATCAGAGT-3′; reverse: 5′-ATATAAACGCCCATAGCTTGCTTAC-3′). After performing a validation experiment to demonstrate that efficiencies of target and reference are approximately equal, the comparative C_T method was used for calculation of *dYARS* expression levels in experimental samples relative to control samples.

Protein Production, Purification, and Aminoacylation Analysis. Full-length WT and mutant *YARS* cDNAs with a C-terminal 6 histidine tag were cloned into pKEW-108 plasmid and overexpressed in *E. coli* strain BL21-CodonPlus(DE3)-RIL (Stratagene) by induction with 1 mM isopropyl β-D-thiogalactopyranoside for 4 h. The proteins were purified from the supernatant of lysed cells under native conditions using a Ni-NTA affinity resin (Qiagen). Protein concentration was determined using the Bradford assay (Bio-Rad) with BSA as a standard. The aminoacylation assay was performed at 37 °C in a 80-μL reaction mixture containing 100 mM Hepes (pH 7.5), 20 mM KCl, 10 mM MgCl2, 2 mM DTT, 2 mM ATP, 2.9 μM L-[3H]-tyrosine, and 100 μM bulk calf liver tRNA (EMD Biosciences and Novagen). Reactions were initiated by addition of 1 μM purified recombinant protein. Aliquots were removed at 6 appropriate time intervals, spotted into filter discs presoaked with 5% trichloroacetic acid, 100 μM L-Tyr, washed 3 times with cold 5% trichloroacetic acid, dried, and measured by scintillation counting (1). Radioactive measurements from an enzyme-free experiment were subtracted from all experimental data.

Luciferase Assay. Luciferase was measured using the Promega Steady-Glo Luciferase Assay Kit, as described in ref. 2. Briefly, elav-GAL4 and nsyb-GAL4 driver lines were crossed to attP16 UAS::Luciferase flies, and flies carrying the attP16 UAS::Luciferase transgene alone were used as background controls. Three adult female flies were collected in 200 μL Promega Glo Lysis Buffer for each sample, and 5 independent samples were collected for each genotype. Flies were homogenized, incubated at room temperature for 10 min, centrifuged for 5 min, and 150 μL supernatant was transferred to a new tube. For luciferase assays, 20 μL of each sample was transferred to a white-walled 96-well plate at room temperature, 20 μL Promega Luciferase Reagent was added to each well and plates were incubated in the dark for 10 min. Luminescence was measured in a Luminoskan Ascent Luminometer (Thermo Labsystems). The obtained values were normalized to total protein concentration, measured using the Pierce BCA Protein Assay Kit. Subsequently, relative luciferase activity compared with attP16 UAS::Luciferase alone (normalized to 1.00 ± SEM) was calculated.

Data S1. We first expressed WT or mutant *YARS* in all tissues using the strong GAL4 driver lines tubulin-GAL4 or actin5C-GAL4^{strong}. Expression of *YARS_E196K* resulted in full developmental lethality, as no adult *YARS_E196K* expressing flies eclosed. Expression of comparable levels of *YARS_G41R* or *YARS_153-156delVKQV* resulted in normal adult offspring frequencies, but expression of higher levels (2 copies of transgene) resulted respectively in full and partial developmental lethality. In contrast, either moderate or high levels of *YARS_WT* resulted in normal adult offspring frequencies. To overcome the full developmental lethality induced by ubiquitous expression of *YARS_E196K*, transgene expression levels were reduced by raising flies at lower temperature (18 °C) or using weaker ubiquitous drivers (daughterless-GAL4 and actin5C-GAL4^{weak}). These strategies indeed resulted in partial developmental lethality, showing that *YARS_E196K* induced developmental lethality is dosage-dependent, as is the case for *YARS_G41R* and *YARS_153-156delVKQV*. Partial developmental lethality could also be induced for *YARS_G41R* expression by using actin5C-GAL4^{strong} to drive expression of one strong and one weak *YARS_G41R* transgene (Table S1). Expression of WT or mutant *dYARS* induced developmental lethality in a similar way as *YARS* expression (Table S2).

Data S2. Flies carrying the actin5C-GAL4^{strong} driver alone also displayed a small but statistically significant motor performance defect (average time in seconds: 5.37 ± 0.06 for actin5C-GAL4^{strong} flies versus 4.53 ± 0.11 for controls, $n = 10$, $P = 2.87 \times 10^{-6}$).

Data S3. Two different deficiency lines uncovering *dYARS* were crossed to Canton-S (B) flies to generate hemizygous *dYARS* flies and genetic controls. Negative geotaxis assays performed on these flies did not reveal any defects in climbing behavior (average time in seconds: 3.99 ± 0.31 for Df(3L)Exel6129/+ flies versus 4.29 ± 0.17 for TM6B/+ controls, $n = 10$, $P = 0.17$; 4.00 ± 0.11 for Df(3L)st-f13/+ flies versus 4.28 ± 0.12 for TM6B/+ controls, $n = 10$, $P = 0.07$).

Data S4. Specific expression of high levels of *YARS_E196K* in muscle (MHC-GAL4) did not impair motor performance (average time in seconds: 3.96 ± 0.07 s for MHC-GAL4 > 2x *YARS_E196K* flies versus 4.08 ± 0.08 s for controls, $n = 10$, $P = 0.31$).

1. Schreier AA, Schimmel PR (1972) Transfer ribonucleic acid synthetase catalyzed deacylation of aminoacyl transfer ribonucleic acid in the absence of adenosine monophosphate and pyrophosphate. *Biochemistry* 11:1582–1589.

2. Markstein M, Pitsouli C, Villalta C, Celniker SE, Perrimon N (2008) Exploiting position effects and the gypsy retrovirus insulator to engineer precisely expressed transgenes. *Nat Genet* 40:476–483.

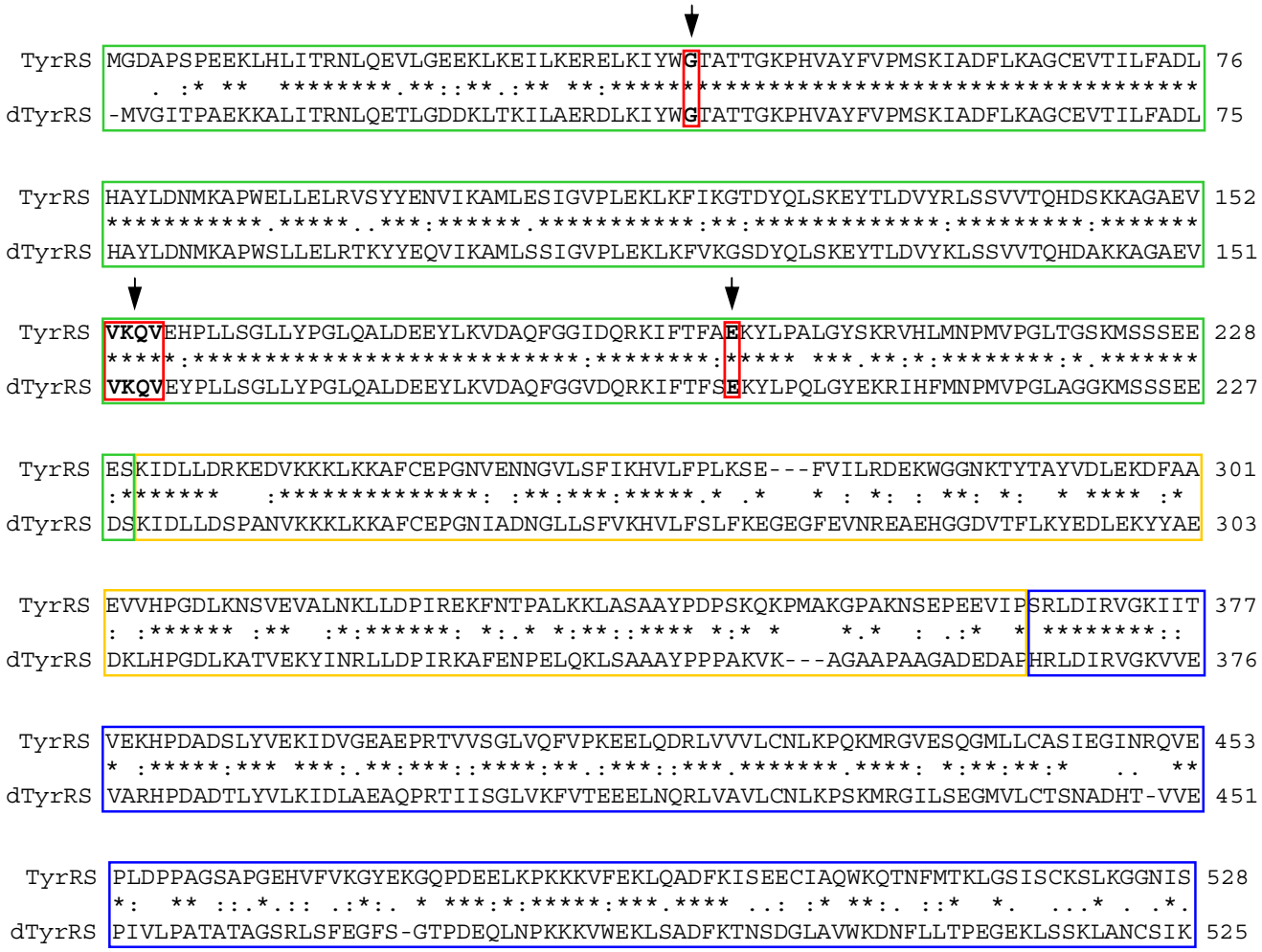


Fig. S1. Alignment of the amino acid sequences of human TyrRS with *Drosophila* TyrRS (dTyrRS) using the ClustalW2 program. The N-terminal catalytic domain (green), the anticodon recognition domain (yellow) and the C-terminal domain (blue) are indicated. Amino acid residues that are mutated in DI-CMTC are in red boxes and indicated by arrows. ''*'' indicates identical residues; '':'' indicates conservative substitutions; and ''.'' indicates semiconservative substitutions.

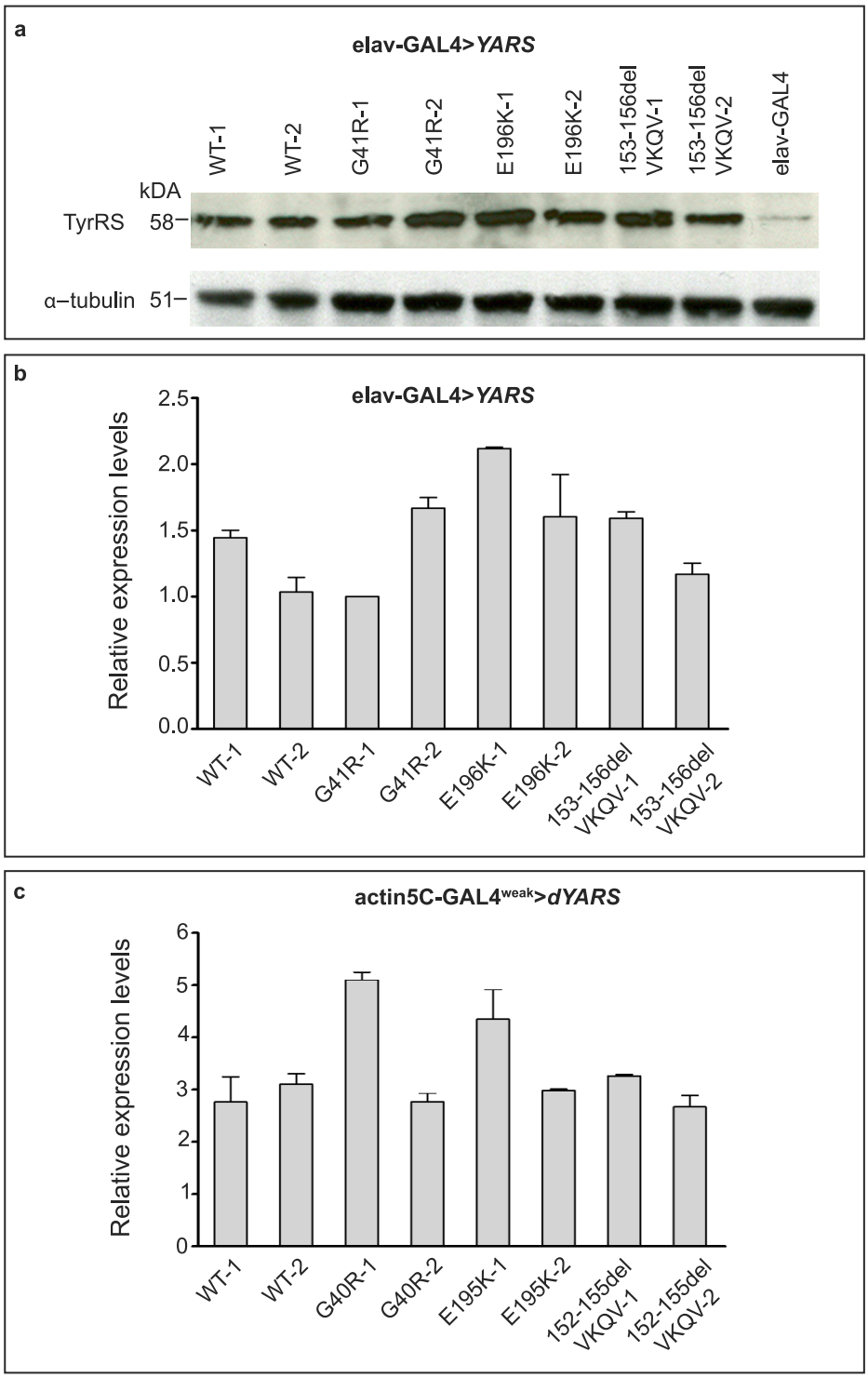


Fig. S2. Determination of *YARS* transgene expression levels. (*a*) Western blot analysis on protein extracts from heads of elav-GAL4 > *YARS* flies with antibodies against TyrRS and α-tubulin as loading control. For each transgene *YARS* expression levels were determined for 2 independent transgenic lines. Head extracts from elav-GAL4 flies were used as negative control. (*b*) Band intensities were quantified, and the ratio of intensities of TyrRS and α-tubulin bands were used to calculate relative expression levels. Lines with similar expression levels (*WT-1*, *G41R-2*, *E196K-2*, and *153-156delVKQV-1*) were selected for further experiments. (*c*) Quantitative real-time PCR on cDNA derived from whole bodies of actin5C-GAL4^{weak} > *dYARS* flies was used to determine relative expression levels of *dYARS* transgenes. For each transgene (*dYARS_WT*, *dYARS_G40R*, *dYARS_E195K*, and *dYARS_152-155delVKQV*), 2 independent transgenic lines were tested. Lines with similar expression levels (*WT-1*, *G40R-2*, *E195K-2*, and *152-155delVKQV-2*) were selected for further experiments.

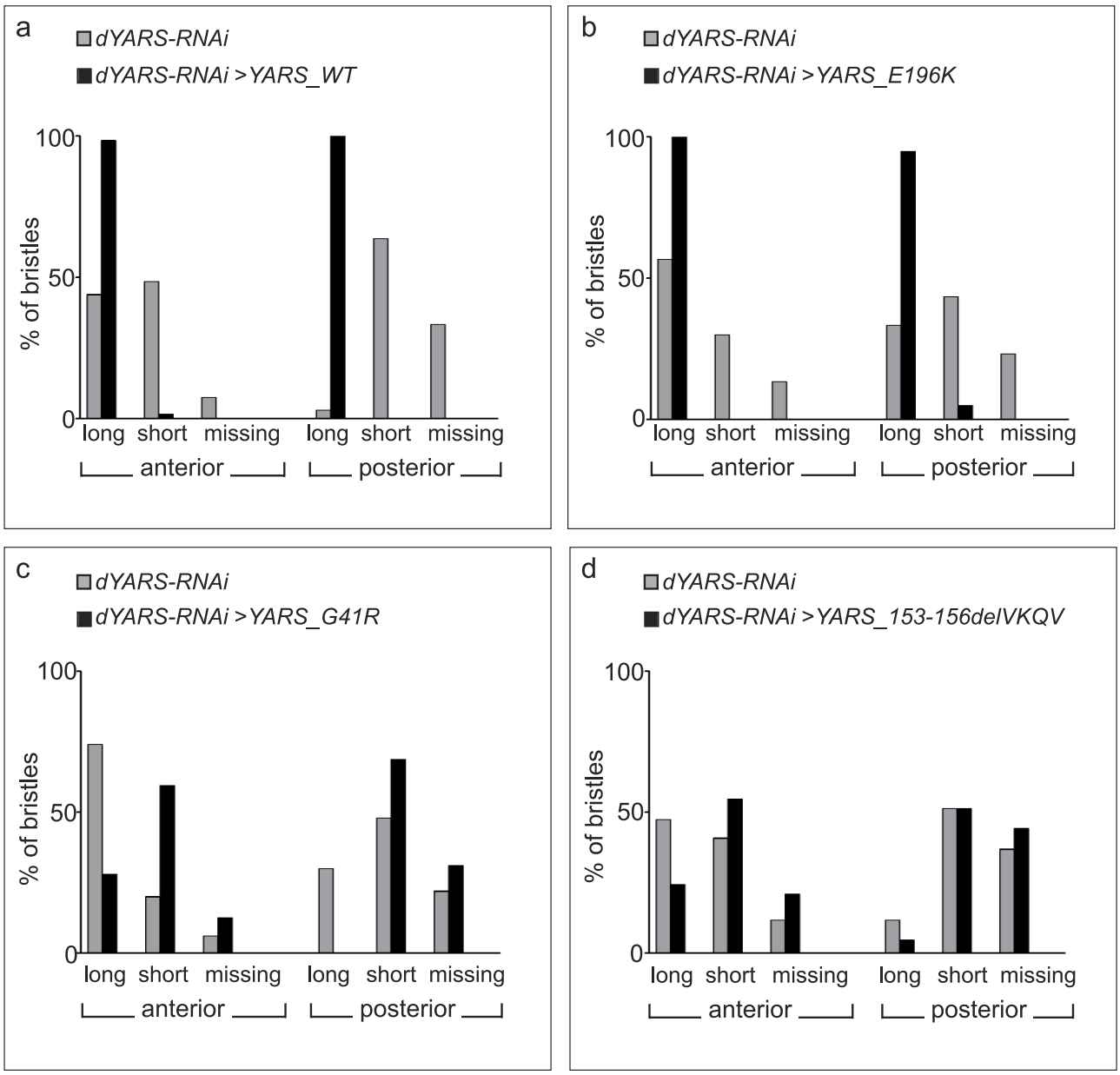


Fig. S3. Effect of coexpression of WT or mutant *YARS* on *dYARS-RNAi* induced bristle phenotypes. (*a–d*) Bar graphs representing the percentage of normal (long), short or missing anterior and posterior scutellar bristles with or without coexpression of *YARS_WT* (*a*), *YARS_E196K* (*b*), *YARS_G41R* (*c*), or *YARS_153-156delVKQV* (*d*). Coexpression of *YARS_WT* and *YARS_E196K* results in full rescue of bristle phenotypes, whereas coexpression of *YARS_G41R* and *YARS_153-156delVKQV* did not rescue bristle phenotypes. More than 30 female flies per genotype were analyzed.

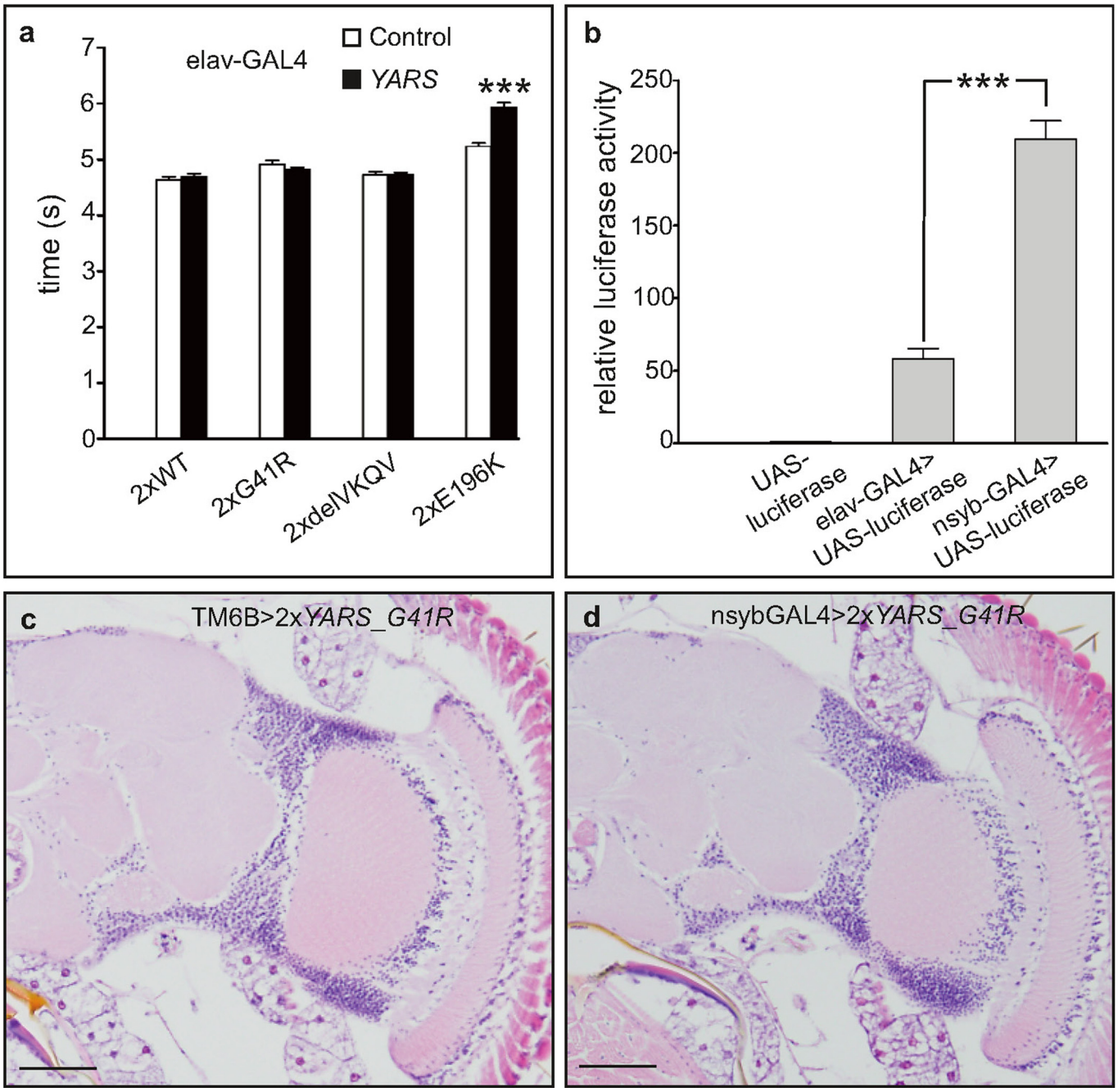


Fig. S4. Neuron-specific expression of mutant *YARS* in *Drosophila* induces impaired motor performance without overt neurodegeneration. (*a*) Negative geotaxis assay on elav-GAL4 > 2× *YARS* flies revealed impaired motor performance in flies expressing *YARS_E196K* panneuronally. (*b*) Luciferase activity in flies expressing a UAS::luciferase transgene driven by elav-GAL4 or nsyb-GAL4, relative to luciferase activity in flies carrying the UAS::luciferase transgene alone. This assay revealed that nsyb-GAL4 drives almost 4 times stronger transgene expression than elav-GAL4. (*c* and *d*) Hematoxylin and eosin staining on brain sections of nsyb-GAL4 > mutant *YARS* flies did not reveal obvious neurodegeneration, which is typically characterized by brain vacuolization in *Drosophila*. Sections through nsyb-GAL4 > 2× *YARS_G41R* (*d*) and control (TM6B > 2× *YARS_G41R*) brains (*c*) are shown. (Scale bar, 50 μm.) ***, $P < 0.001$; indicates a significant difference versus genetic controls, as determined by one-way ANOVA with Bonferroni's Multiple Comparison Test.

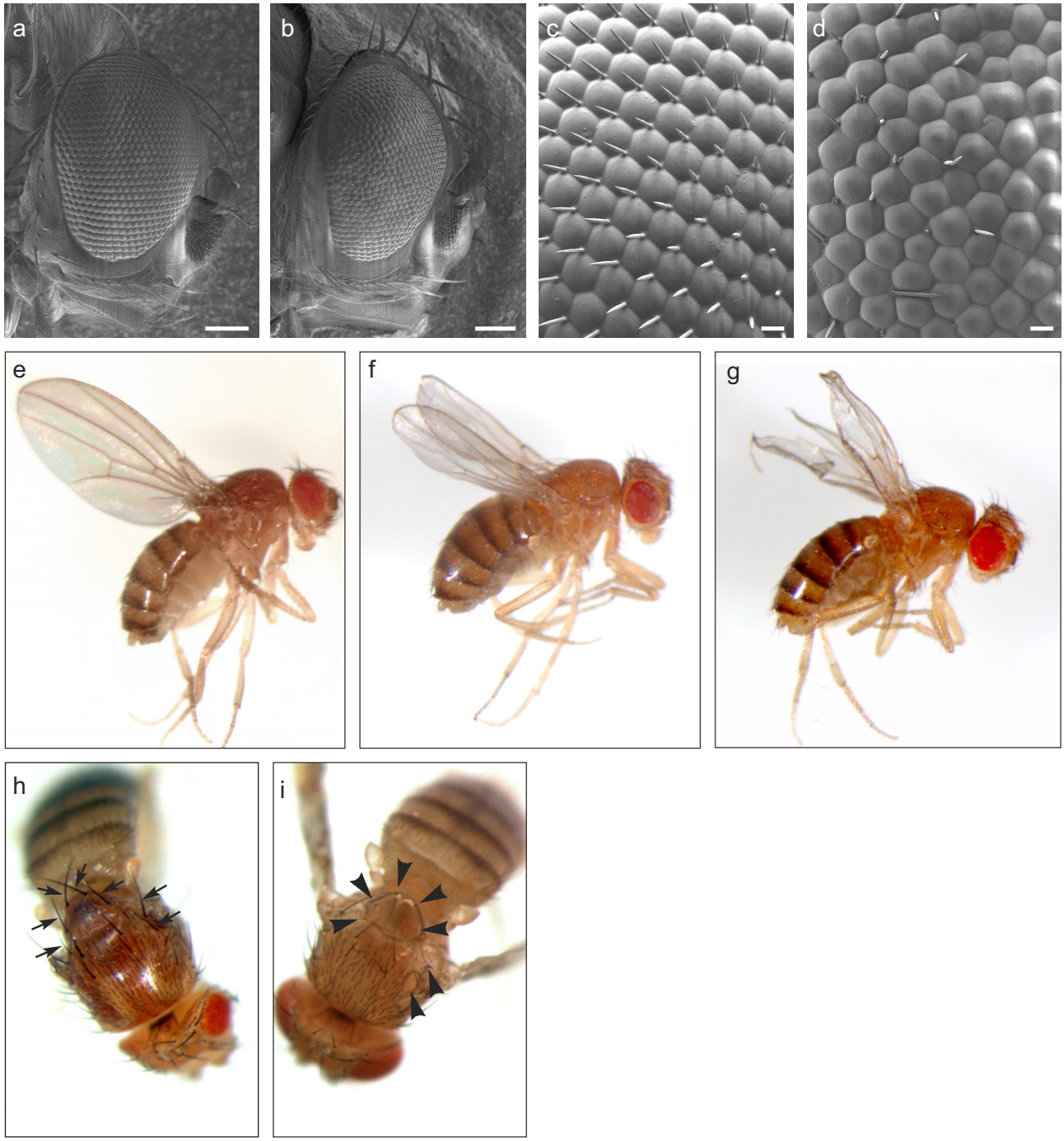
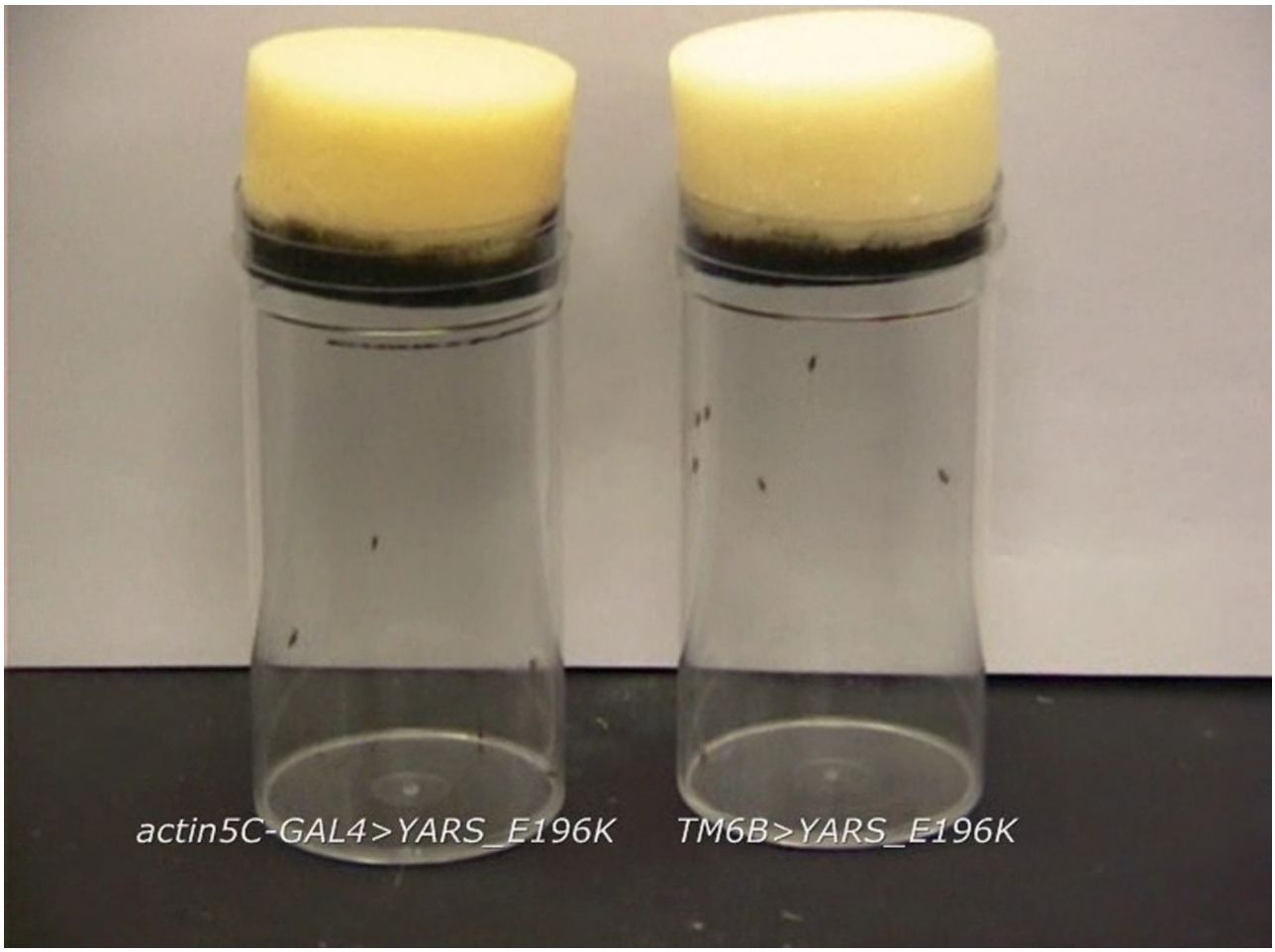
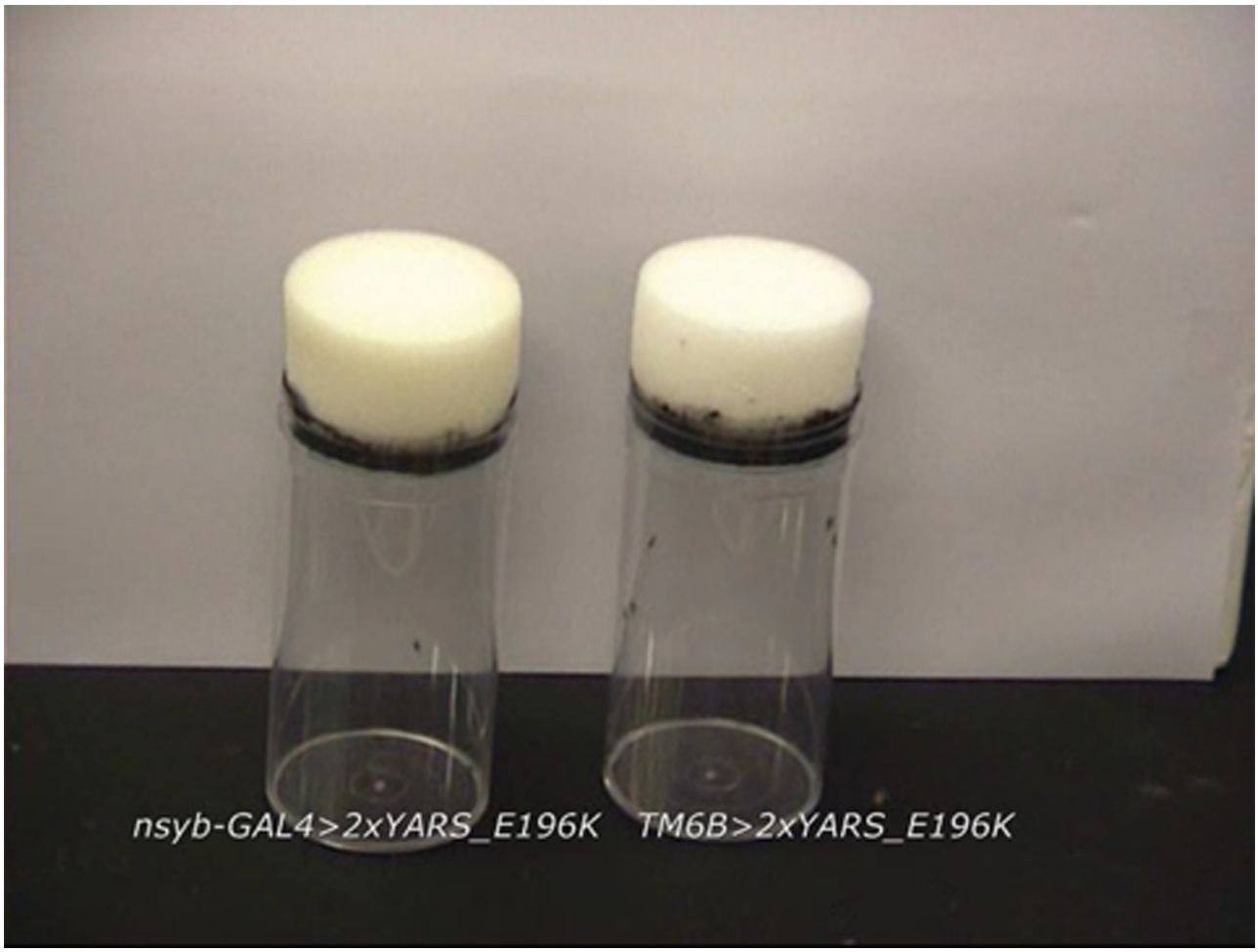


Fig. S5. Phenotypes induced by tissue-specific expression of *YARS* in *Drosophila*. (*a*–*d*) GMR-GAL4 driven expression of *YARS_E196K* induces eye phenotypes. (*a* and *b*) Scanning EM pictures of eyes of flies expressing *YARS_E196K* in the retina (*b*) (GMR-GAL4/*YARS_E196K; YARS_E196K*/+) and genetic controls (*a*) (GMR-GAL4/+). (Scale bar, 100 μm.) (*c* and *d*) higher power images of *YARS_E196K* (*d*) and control eyes (*c*) showing the irregular shape and nonuniform size of ommatidia in *YARS_E196K* eyes, in contrast to the extremely regular hexagonal shape and uniform size of ommatidia in genetic controls. (Scale bar, 10 μm.) (*e*–*i*) Apterous-GAL4 driven expression of *YARS_E196K* induces wing and bristle phenotypes. (*e*–*g*) Compared with genetic controls (e) (*L*/+; *YARS_E196K*/+), apterous-GAL4 > *YARS_E196K* wings looked fragile, often had a curly appearance (*f*) and were occasionally damaged (*g*). (*h* and *i*) Thoracic bristles of apterous-GAL4 > *YARS_E196K* flies were often shorter or missing (*i*), and this was never the case in genetic controls (*h*).



Movie S1. *YARS_E196K* expressing flies display impaired motor performance. In a negative geotaxis assay, the average time needed to reach the top of the vial was almost doubled for actin5C-GAL4^{weak} > *YARS_E196K* flies (left vial) compared with TM6B > *YARS_E196K* controls (right vial).

Movie S1 (WMV)



Movie S2. Panneuronal expression of *YARS_E196K* results in impaired motor performance. In a negative geotaxis assay, nsyb-GAL4 > 2× *YARS_E196K* flies (left vial) are unable to reach the top of the vial, in contrast to TM6B > 2× *YARS_E196K* control flies (right vial).

Movie S2 (WMV)

Table S1. Adult offspring frequencies of *YARS* expressing flies

Driver	Transgene	Control	*YARS*	*P* value
tubulin-GAL4	*YARS_WT* line 1	92	107	0.29
tubulin-GAL4	*YARS_WT* line 2	72	94	0.088
actin5C-GAL4^{strong}	2x *YARS_WT*	604	545	0.082
tubulin-GAL4	*YARS_E196K* line 1	395	0	6.75×10^{-88}
tubulin-GAL4	*YARS_E196K* line 2	80	0	3.74×10^{-19}
tubulin-GAL4	*YARS_G41R* line 1	86	111	0.075
tubulin-GAL4	*YARS_G41R* line 2	73	82	0.47
actin5C-GAL4^{strong}	2x *YARS_G41R*	342	0	2.34×10^{-76}
tubulin-GAL4	*YARS_153–156delVKQV* line 1	97	125	0.060
tubulin-GAL4	*YARS_153–156delVKQV* line 2	50	52	0.84
actin5C-GAL4^{strong}	2x *YARS_153–156delVKQV*	309	191	1.31×10^{-7}
tubulin-GAL4$^{18\ °C}$	*YARS_E196K* line 1	213	13	2.20×10^{-40}
tubulin-GAL4$^{18\ °C}$	*YARS_E196K* line 2	102	8	3.17×10^{-19}
daughterless-GAL4	*YARS_E196K* line 1	305	71	1.57×10^{-33}
daughterless-GAL4	*YARS_E196K* line 2	109	27	2.04×10^{-12}
actin5C-GAL4^{weak}	*YARS_E196K* line 1	125	43	2.51×10^{-10}
actin5C-GAL4^{weak}	*YARS_E196K* line 2	115	42	5.68×10^{-9}
actin5C-GAL4^{strong}	*YARS_G41R^{strong} > YARS_G41R^{weak}*	354	110	9.60×10^{-30}

The number of adult flies eclosing is indicated for each genotype.

Table S2. Adult offspring frequencies of *dYARS* expressing flies

Driver	Transgene	Control	*dYARS*	*P* value
actin5C-GAL4^{strong}	*dYARS_WT* line 1	293	293	1
actin5C-GAL4^{strong}	*dYARS_WT* line 2	265	282	0.47
actin5C-GAL4^{strong}	2x *dYARS_WT*	308	262	0.054
actin5C-GAL4^{strong}	*dYARS_E195K* line 1	449	0	1.19×10^{-99}
actin5C-GAL4^{strong}	*dYARS_E195K* line 2	547	0	5.66×10^{-121}
actin5C-GAL4^{strong}	*dYARS_G40R* line 1	314	308	0.81
actin5C-GAL4^{strong}	*dYARS_G40R* line 2	227	217	0.64
actin5C-GAL4^{strong}	2x *dYARS_G40R*	155	0	1.40×10^{-35}
actin5C-GAL4^{strong}	*dYARS_152–155delVKQV* line 1	237	272	0.12
actin5C-GAL4^{strong}	*dYARS_152–155delVKQV* line 2	276	299	0.34
actin5C-GAL4^{strong}	2x *dYARS_152–155delVKQV*	325	108	1.84×10^{-25}
actin5C-GAL4^{weak}	*dYARS_E195K* line 1	325	68	1.96×10^{-38}
actin5C-GAL4^{weak}	*dYARS_E195K* line 2	340	75	1.09×10^{-38}
actin5C-GAL4^{strong}	*dYARS_G40R^{strong} >dYARS_G40R^{weak}*	246	149	1.06×10^{-6}

The number of adult flies eclosing is indicated for each genotype.

Table S3. Jumping and flying ability of *YARS* expressing flies

Driver	Transgene	Jump, %	Fly, %	Veer, %	Fall, %
actin5C-GAL4^{strong}	2x *YARS_WT*	100	100	0	0
actin5C-GAL4^{weak}	*YARS_E196K*	70	8	35	57
actin5C-GAL4^{strong}	*YARS_G41R^{strong} > YARS_G41R^{weak}*	92	55	33	12
actin5C-GAL4^{strong}	2x *YARS_153–156delVKQV*	100	88	6	6

Fifty 23-day-old female flies of each genotype were assayed. Control flies lacking the driver were always able to jump and to fly.